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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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Name

学位論文題目： **Study of Allelopathic Potential and Allelopathic Substances of Four Weed Species in Crop Fields**
Title of Dissertation **（農地における雑草４種のアレロパシー活性とアレロパシー候補物質の研究）**

学位論文要約：
Dissertation Summary

Weeds are plant species on cultivated land which are unwanted. There are approximately 8000 weed species globally, of which 80 weed species are known to reduce the crop yields (Singh *et al*., 2001). They deprive the crop plants from limited available nutrients, space, light, and moisture, which results in a reduction of crop yield and quality (Singh *et al*., 2001; Khanh *et al*., 2007). Thus, farmers use synthetic herbicides to control weed. Overuse of synthetic herbicides for controlling weeds can risk the development of weed biotypes being resistant to herbicides, in addition to raising concerns about environmental pollution (Beckie, 2006; Awan *et al*., 2015). Also, some weed control tactics such as cultural, mechanical are not cost-effective and durable (Jabran *et al*., 2015). So, sustainable cost effective and eco-friendly weed management strategies place demands on time across the globe (Bajwa *et al*., 2015; Amb and Ahluwalia, 2016). Using allelopathy and/or potential allelopathic plants in weed management means that sustainable weed control can address the challenges of herbicide resistance development and environmental pollution.

Allelopathy is a term coined by Hans Molisch, in 1937, using the Greek words "allelon", meaning of each other, and "pathos", meaning to suffer the injurious effect of one on another. Rice (1984) refined the term as “any direct or indirect harmful or beneficial effect by one plant (including microorganisms) on another through production of chemical compounds that escape into the environment” (Rice, 1984; Cheng and Cheng, 2015). Allelochemicals are secondary metabolites and found in leaves, stems, roots, rhizomes, seeds, flowers and even pollen in different concentrations. They are released into the environment via different pathway such as: (1) exudation and deposition on the leaf surface with subsequent washing off by rainfall, (2) exudation of volatile compounds from living green parts of the plant, (3) decay of plant residues (litter fall or dead roots), and (4) root exudation (Chon *et al*., 2006; Sangeetha and Baskar, 2015). Allelochemicals released into the environment have influences from the plant itself and several biotic and abiotic factors (de Albuquerque *et al*., 2011**)**. Also, allelochemicals production varies in plant species and age, and tissue type (Latif *et al*., 2017).

Allelopathy is important for agricultural practices as has gained attention in weed control because of concerns relating to synthetic herbicide use and increased amounts of herbicide resistant weed biotypes (Beckie, 2006; Awan *et al*., 2015). Allelopathy provides weed control through producing and releasing allelochemicals. Allelopathy for controlling weeds is either through directly utilizing natural allelopathic interactions or by using allelochemicals as natural herbicides (Bhowmik and Inderjit, 2003; Jabran *et al*., 2015). A number of plants, including crops, weeds and trees, have been reported to have allelopathic properties, and an estimated 240 species of weeds possess allelopathic potential (Qasem and Foy, 2001). Studies have proven that weed species have allelopathic potential and a number of allelopathic substances reportedly also inhibit the growth of other plants (Islam *et al*., 2014; Hegab *et al*., 2016; Islam *et al*., 2017a, 2017b, 2017c). Therefore, this study explores the allelopathic potentiality of four weed species in crop fields, and for isolating and identifying allelopathic substances therein.

In the study four weed species such as *Cyanotis axillaris* L. (Commelinaceae), *Paspalum commersonii* Lam. (Poaceae), *Oxalis europea* L. (Oxalidaceae) and *Eleocharis atropurpurea* Retz. (Cyperaceae) were chosen (Figure 1). In Bangladesh, all these weed species are often appears in the rice, jute and wheat fields and are competitive weeds as their management is troublesome. *Cyanotis axillaris* is a prostrate and creeping type annual weed. It grows up to 90 cm long and rooting at the nodes. The stems are succulent, solid and rounded (Gupta, 2011). *Paspalum commersonii* is an herbaceous perennial weed found in the tropics and subtropics. *P. commersonii* often appears in the rice field as a competitive weed and is difficult to manage. It usually grows in the moist, or even flooded soil (Hsu *et al*., 2000). *Oxalis europea* is an annual weed found largely in tropical and subtropical regions. It is a weed found particularly in wheat fields. *O. europea* leaves contain oxalic acid and pleasant sour in taste. They prefer to grow in sandy and loamy soils as well as well drained soil, rather than in shady areas (Clapham *et al*., 1962; Usher, 1974). On the other hand, *Eleocharis atropurpurea* is an annual sedge, featuring green, tufted, erect and slender stems. The species prefers marshes, shallow water, river beds and flooded places for growth, as well as growing in rice fields (Cook, 1996; Kumar, 2014). However, to the best of our knowledge, no research has yet been found on the allelopathic potential of *C. axillaris*, *P. commersonii*, *O. europea* and *E. atropurpurea* as well as their allelopathic substances.

C. axillaris **L.** ***P. commersonii*** **Lam.** ***O. europea*** **L.** ***E. atropurpurea*** **Retz.**

Figure 1. Four weed species used as research materials.

Plant powder of each weed species (*C. axillaris*, *P. commersonii*, *O. europea* and *E. atropurpurea*) was extracted with 70% (v/v) aqueous methanol and methanol. The crudes were dissolved in methanol to prepare six bioassay concentrations (0.001, 0.003, 0.01, 0.03, 0.1 and 0.3 g dry weight equivalent extract/mL). Eight test species were selected for bioassay test plant species: the dicotyledons cress (*Lepidium sativum* L.), rapeseed (*Brassica napus* L.), alfalfa (*Medicago sativa* L.) and lettuce (*Lactuca sativa* L.), and the monocotyledons Italian ryegrass (*Lolium multiflorum* Lam.), barnyard grass (*Echinochloa crus-galli* (L.) P. Beauv.), foxtail fescue

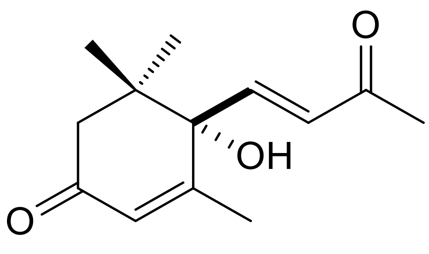
(*Vulpia myuros* (L.) C. C. Gmel.) and timothy (*Phleum pretense* L.). These test species are commonly used for laboratory bioassay.

The results showed that aqueous methanol extracts of *C. axillaris*, *P. commersonii*, *O. europea* and *E. atropurpurea* showed concentration-dependent inhibitory activity on both monocotyledonous (Italian ryegrass, barnyard grass, foxtail fescue, timothy) and dicotyledonous (cress, rapeseed, alfalfa, lettuce) test plant species. The *C. axillaris* extracts at 0.3 g dry weight equivalent extract/mL completely inhibited the shoot and root growth of cress, rapeseed, alfalfa, lettuce, while at the same concentration, other test plant species showed inhibition greater than 30% of control shoot and root growth. The extracts obtained at a concentration of 0.3 g dry weight equivalent extracts of *P. commersonii*/mL completely inhibited the shoot and root growth of cress and the root growth of lettuce and foxtail fescue. At the same concentration, the shoot growth of rapeseed, alfalfa, lettuce, Italian ryegrass, barnyard grass, foxtail fescue and timothy were inhibited by 1.5, 0.8, 0.7, 1.5, 20.3, 8.9 and 0.5% of the control shoot growth, respectively, and the root growth of rapeseed, alfalfa, Italian ryegrass, barnyard grass and timothy were inhibited by 0.8, 0.8, 0.7, 1.6 and 0.5% of the control root growth, respectively. On the other hand, the aqueous methanol *O. europea* extracts inhibited the seedling growth of cress, rapeseed, alfalfa, lettuce, Italian ryegrass, barnyard grass, foxtail fescue, timothy at concentrations of more than 0.01 g dry weight equivalent extract/mL. The concentration of 0.1 g dry weight equivalent extract of *E. atropurpurea*/mL completely inhibited the shoot and root growth of cress and lettuce. At 0.3 g dry weight equivalent extract of *E. atropurpurea*/mL, rapeseed also showed complete growth inhibition, whereas other test plant species showed inhibition greater than 25% of control growth. The concentration-dependent inhibitory activity of the extracts concurs with the findings of Grisi *et al*. (2012), who found concentration dependent inhibitory effects on the seedling growth of *Lactuca sativa* by the extracts of *Sapindus saponaria*.

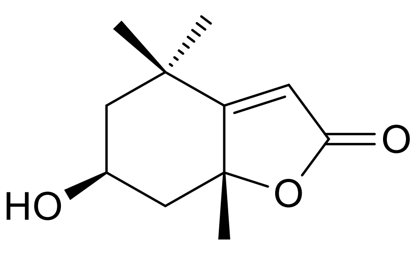
The I_{50} values (concentration required for 50% growth inhibition) of the extracts of *C. axillaris*, *P.*

commersonii*, *O. europea* and *E. atropurpurea* were different for shoot and root growth of the test plants and the values ranged from 0.003 to 0.036, 0.002 to 0.085, 0.002 to 0.026 and 0.002 to 0.097 g dry weight equivalent extract/mL, respectively. These results also indicaing that inhibition was also depended on the test plant species. The concentration- and test plant-dependent inhibitory activity of the extracts of *C. axillaris*, *P. commersonii*, *O. europea* and *E. atropurpurea* indicate that all of these weeds have allelopathic potential and may contain allelopathic substances.

The concentrated residue was then adjusted to pH 7.0 with NaOH (1 N) and partitioned with an equal volume of ethyl acetate to get ethyl acetate and aqueous fraction. The aqueous and ethyl acetate fractions showed concentration-dependent inhibitory activities on the shoot and root growth of cress. The ethyl acetate fraction was purified using a silica gel column, Sephadex LH-20 column, C_{18} cartridge and HPLC. Two substances were isolated from *P. commersonii* extracts and characterized as dehydrovomifoliol and loliolide by using HRESIMS, ^{1}H NMR (Figure 2). Dehydrovomifoliol significantly inhibited the shoot and root growth of cress seedlings at concentrations greater than 3 and 3.5 mM, respectively. On the other hand, loliolide inhibited cress seedling growth at 0.03 mM, and the inhibition was 53.0–61.5% of the control seedling growth. The inhibition increased with increasing concentrations of dehydrovomifoliol and loliolide. However, to the best of our knowledge, this study is the first to report on isolating these two substances from *P. commersonii*.



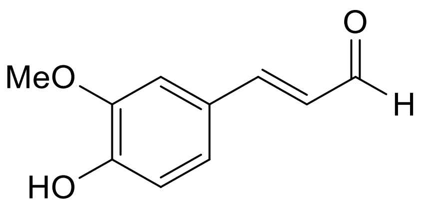
Dehydrovomifoliol



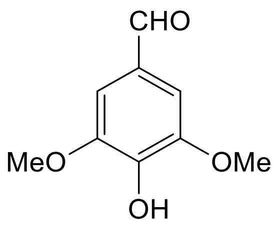
Loliolide

Figure 2. Chemical structure of the isolated substances from the extracts of *P. commersonii*.

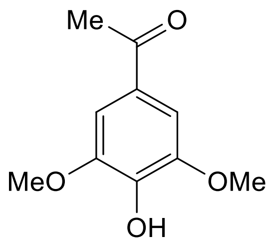
Three substances were isolated from the extracts of *E. atropurpurea* and characterized as *trans*-ferulaldehyde, syringaldehyde and acetosyringone by using HRESIMS, ^{1}H NMR (Figure 3). Every compounds have activity against seedling growth of cress and barnyard grass at different levels. The I_{50} values of *trans*-ferulaldehyde, syringaldehyde and acetosyringone for the shoot and root growth of cress and barnyard grass were ranged 0.73 to 4.54 mM. Comparing I_{50} values, cress seedling was more sensitive to the compounds than was barnyard grass. To the best of our knowledge, this study is the first report on the inhibitory activities of *trans*-ferulaldehyde, syringaldehyde and acetosyringone from *E. atropurpurea* weed.



trans-Ferulaldehyde



Syringaldehyde



Acetosyringone

Figure 3. Chemical structure of the isolated substances from the extracts of *E. atropurpurea*.

This study concludes that, the aqueous methanol extracts of *Cyanotis axillaris*, *Paspalum commersonii*, *Oxalis europea* and *Eleocharis atropurpurea* showed concentration- and species- dependent inhibitory activity on the seedling growth of the test plants, which indicates that four weed species might possess allelopathic potential and allelopathic substances. Five allelopathic substances were isolated from the extracts of *P. commersonii* and *E. atropurpurea* and these substances displayed inhibitory activity on cress and barnyard grass seedling growth. To the best of our knowledge, this study is the first to report the allelopathic potential of these weed species. Therefore, the allelopathy of *C. axillaris*, *P. commersonii*, *O. europea* and *E. atropurpurea* weeds might have a crucial role in developing alternative weed management strategies.

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